2401-Pos Board B387
A Model for the Motion of Listeria in Curved Paths
Yuan Lin, Vivek Shenoy.
The movement of certain pathogens, such as Listeria monocytogenes, is commonly believed to be driven by actin polymerization. By hijacking the actin machinery of the host cell, these pathogens can move in the cytoplasm with a speed in the order of 0.1 μm/s. Several microscopic or macroscopic models have been proposed to account for the force generation by actin polymerization, however, a theoretical explanation for the rich trajectories traced out by the pathogens, including circles, winding S curves, translating figure eights, and serpentine shapes etc., is still lacking.
Here we show that the non-uniform polymerization of actins behind Listeria could be responsible for these fascinating trajectories. Specifically, we will demonstrate that if polymerization is fast at one side of the comet tail and slow at the other, then Listeria will move in circles. Stress profiles corresponding to different polymerization conditions will also be presented and, interestingly, we found that, depending on the degree of the non-uniformity in polymerization rate, some actin filaments might generate propelling forces pushing the bacteria forward while others might exert forces opposing the movement of Listeria, which is consistent with recent experimental observations.
In addition to a propelling force normal to the load surface, our model also predicts the generation of a tangential force by polymerizing filament. Consequently, a torque can be generated within the comet tail, causing Listeria to spin, if the symmetry of the actin network is broken. We will further demonstrate how various trajectories observed in experiments, such as winding S curves and translating figure eights, can be related to the asymmetric structure of the actin network, as well as the non-uniform polymerization of actins.

2402-Pos Board B388
Micro-Fabricated Substrates to Study Mechanotransduction
Jimmy Le Digabel.
Cellular processes imply an important coordination of interactions with the extracellular medium. Accumulating evidences demonstrate that cell functions can be modulated by physical factors such as the mechanical forces acting on the cells and the extracellular matrix, as well as the topography or rigidity of the matrix. These extracellular signals can be sensed by mechanosensors on the cell surface or in the cell interior to induce various cell responses. We have developed an original approach based on micro-fabricated substrates of PolyDimethylSiloxane (PDMS) to study cell migration. We used a closely spaced array of flexible micropillars of different sizes to measure the forces exerted by cells on their substrates and to modify the effective rigidity of the substrate. In particular, we performed durotaxie experiments using substrates with a well defined frontier between two regions of different rigidities. We observed that a cell arriving to the frontier is more inclined to go to the more rigid region than to the softer one. We propose also to analyze the cell response to an external applied stress by a well-controlled actuation of the substrate. To do so, we developed magnetic pillars. Using polyacrylamide hydrogels doped with ferromagnetic iron oxide particles or ferrofluids, we can make magnetic pillars with diameters of 4 to 10 μm while a magnetic field can be locally applied with a magnetic needle. With such a technique we can exert forces of the range of several nN. Those substrates can be helpful to study the mechanical response of cells to an external force or to local changes in their microenvironment.

2403-Pos Board B389
Controlling Dorsal Ruffles in Cells Through Substrate Stiffness and Mechanical Stimulation
Yukai Zeng, Philip R. LeDuc, Keng Hwee Chiam.
Circular dorsal ruffles (CDRs) are actin rich structures formed in mammalian cells in response to stimulation with growth factors, such as platelet-derived growth factor (PDGF). CDR formation is a possible mechanism by which the actin cytoskeleton in cells is remodeled relating to migration. We have found that CDRs form immediately after PDGF stimulation, before persisting and disappearing after tens of minutes in fibroblast cells seeded on underlying polydimethylsiloxane (PDMS) substrates coated with fibronectin. Increasing the PDMS stiffness with the same PDGF stimulation in NIH 3T3 fibroblasts increases the percentage of cells in a population which contain CDRs. Increasing PDMS substrate stiffness also results in a biphasic response of the average persistence time of CDRs formed in a cell population. This biphasic response reveals that the percentage of cells with CDRs increases at low stiffnesses while decreasing at higher stiffnesses. Not only does the substrate stiffness affect the CDRs, but by applying biaxial stresses to the cells through stretching the PDMS substrates, we observe an increase in the average persistence time of CDRs formed in a cell population with similar PDGF stimulation. Our results suggest that the functionality of CDRs in cells after stimulation with PDGF is linked to substrate stiffness and also the mechanical environment of the cells. We believe that this work is of interest to a wide variety of fields including physics, biology, material science, and mechanics.

2404-Pos Board B390
The Mechanism of Gut Loops Morphogenesis
Thierry Savin.
The small intestine has a remarkably complex morphology. It exhibits a distinguished coiled shape, with a succession of loops and twists. The latter allow for the long transit times required for digestion and nutrients absorption that characterize its function. Given this peculiar configuration, understanding development and morphogenesis of the gut appears to be a challenging, yet important task. I will show how we explain the formation and shape of gut loops by using a simple mechanical model, based on the differential growth between the gut tube and the mesentery, that is the membrane tissue that holds the intestine in place.

2405-Pos Board B391
Mechanical Control of Epithelial Growth: Distinct Morphogenetic Regimes
Oswaldo A. Lozoya, **Sharon R. Lubkin**.
We develop a model of transport and growth in epithelio-mesenchymal interactions. Analysis of the growth of an avascular epithelial spheroid inside a passive mesenchyme shows that sustained volumetric growth requires four generic mechanisms: (1) growth factor, (2) protease, (3) control of cellularity, and (4) swelling. The model reveals a bifurcation delineating two distinct morphogenetic regimes: (A) steady epithelial growth, (B) epithelial growth arrested by capsule formation in the mesenchyme. In both morphogenetic regimes, growth velocity is constant unless and until a complete capsule forms. Comprehensive exploration of the parameter space reveals that the bifurcation is determined by a ratio of the relative strengths of growth and proteolytic activity. Growth velocity is determined only by the strength of growth signaling, independent of proteolytic activity. There is a continuum of bulk versus surface growth, with fastest growth at the surface. The model provides a theoretical basis for explaining epithelial growth arrest despite proteolysis of surrounding tissue, and gives a quantitative framework for the design and interpretation of experiments.

2406-Pos Board B392
Influence of Substrate Thickness and Stiffness on Cell Behavior
Srikanth Raghavan, Aravind R. Rammohan, Martial Hervy.
It is known that various cell types can sense and respond to the mechanical properties of their microenvironment. Specifically, cells have been known to spread more when cultured on stiff substrates and are able to match their internal stiffness to that of the substrate. It has also been recently reported that even some cells are cultured on soft but thin coatings attached to stiff supports, they can perceive an effective stiffness that is much larger than that of the bulk coating. Building on earlier models [1,2], we present a model and invoke arguments based on energetics that explain why stiff and thin substrates encourage some cells to spread more easily and why these cells can match their internal stiffness to external stiffness more efficiently than others.

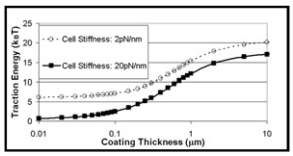

Figure : Traction energy as a function of coating thickness for different cell stiffnesses

Reference:
1. U. S. Schwarz et al, Biosystems, **83**, 225 (2006).
2. J. M. Maloney et al, Phys. Rev. E, **78**, 041923 (2008).

2407-Pos Board B393
Mechanosensing Directs Mitotic Signaling Proteins to Sites of High Mechanical Stress to Regulate Cytokinesis Through a Feedback Control System
Yee Seir Kee, Richard Firtel, Pablo Iglesias, Douglas Robinson.
Cytokinesis is the process by which a cell divides into two daughter cells and must be well regulated to prevent aneuploidy. The mitotic spindle is commonly viewed to initiate cleavage furrow formation through spindle signaling to the polar and furrow cortices. However, a number of studies have shown that the furrow can form independently of the mitotic spindle. Using *Dictyostelium discoideum*, our lab found that cellular mechanosensing is important for cell shape control during cytokinesis. Using micropipette aspiration (MPA) to apply mechanical stress to the cell cortex, we discovered that mechanical stress stimulates accumulation of myosin-II (a force-generating protein) and cortexillin-I (an actin-bundling protein) to the deformation site to correct cell shape. Both myosin-II and cortexillin-I localize to the cleavage furrow and are essential for mechanosensing. Here, we investigated how the spindle signals and mechanosensing work together to control cytokinesis. We found that the kinesin-6-family protein Kif12 can be recruited to mechanically stressed regions in a manner that is dependent on myosin-II and IQGAP2, a signaling effector of cortexillin-I. However, Kif12 is not required for myosin-II mechanosensing. Using agar overlay, we applied a uniform mechanical stress on the cortex. Previous studies showed that dividing cells under this condition have an enhancement of myosin-II localization at the cleavage furrow. In our study, we found

that this enhancement is lost in *kif12* nulls. We propose that spindle signaling proteins, such as Kif12, can be recruited to mechanically stressed region through the mechanosensing system. Upon recruitment to the stressed furrow cortex, spindle signals then may augment the localization of myosin-II to the cleavage furrow. Therefore, the spindle signals and mechanosensing work synergistically as a feedback control system that regulates cytokinesis.

Cytoskeletal Protein Dynamics

2408-Pos Board B394
Pattern Formation in Active Fluids
Justin S. Bois, Frank Jülicher, Stephan W. Grill.
Motivated by the dynamics of the actomyosin cell cortex in which stress generation is under biochemical regulation, we develop a hydrodynamic theory for pattern formation in active fluids. Nonhomogeneous active stress profiles drive fluid motion which transports diffusing stress regulators by advection. Based on this principle, we present a mechanism for pattern formation in which a single diffusing species up-regulates active stress, resulting in steady nonhomogeneous flow and concentration profiles. We also investigate general pattern-forming behaviors of reaction diffusion systems embedded in active fluids. In particular, we find that the presence of active stress-driven flow greatly expands the region of parameter space in which patterns may form in classic Turing-type systems.

2409-Pos Board B395
Traveling Waves and Patches in Dendritic Actin Nucleation
Anders E. Carlsson.
The dynamics of cell edges are closely related to those of F-actin. Recent fluorescence imaging studies have shown that F-actin can spontaneously form traveling waves or moving patches at low actin concentrations. I investigate possible mechanisms for such phenomena by numerically simulating the "dendritic nucleation" model of actin network growth. The simulations treat actin network growth on a 3 by 3 micron piece of membrane. They store information about actin filaments subunit by subunit, giving an explicit three-dimensional picture of the actin network. The calculations include filament growth, capping, branching, severing, and random thermal motion. The dynamics of nucleation-promoting factors (NPFs) in the membrane are also included: they diffuse in the membrane, and detach/inactivate in the presence of F-actin. The simulations show three types of behavior which are "tuned" by the actin concentration: 1) traveling waves, 2) coherently moving patches, and 3) random fluctuations with occasional moving patches. Wave formation is favored by a long recovery time for NPFs which have been inactivated, and by weakness of the attractive interaction between filaments and the membrane. Low G-actin concentrations cause waves to break up into patches which, however, move coherently. Similar effects are seen with increasing capping-protein concentration. Diffusion of NPFs slows the waves, and, if fast enough, stops them completely, resulting in the formation of a static spot.

2410-Pos Board B396
Segmentation and Tracking of Cytoskeletal Filaments Using Open Active Contours
Matthew B. Smith, Hongsheng Li, Tian Shen, Xiaolei Huang, Eddy Yusuf, Dimitrios Vavylonis.
We developed an interactive software tool to quantify cytoskeletal filaments imaged by fluorescence microscopy in two and three dimensions. Our software allows users to visualize and record the position of filaments by implementing a robust algorithm in conjunction with user interaction. We use open active contours for segmentation and tracking of individual fibers. Open active contours are parametric curves that deform to minimize the sum of an external energy derived from the image and an internal bending and stretching energy. The external energy generates (i) forces that attract the contour towards the central bright line of a filament in the image, and (ii) forces that stretch the active contour towards the ends of bright ridges. Images of simulated semiflexible polymers with known bending and torsional rigidity are analyzed to validate the method. Analysis of simulated filaments illustrated the advantages and inherent limitations in representing filaments as a discrete series of points. We apply our methods to quantify the conformations of actin in two examples. We calculated the persistence length of actin filaments imaged by TIRF microscopy in vitro, and we segmented actin cables in fission yeast imaged by spinning disk confocal microscopy.

2411-Pos Board B397
Visualizing the Dynamic Response of the Actin Cytoskeleton
Hedde van Hoorn, Thomas Schmidt.
Cells constantly sense the stiffness of- and forces exerted by their environment. This information activates specific molecular and gene regulation pathways. In order to visualize the dynamic process of mechanotransduction, Photo-Activatable Fluorescent Proteins (PAFPs) are used to label proteins involved in those pathways. Tracking PAFPs gives controlled information on motion of the proteins in space and time. The dynamic response to mechanical cues of the actin cytoskeleton can thus be measured.
Dendra2 is a monomeric green-to-red convertible PAFP, which is especially suitable for use in living cells. Its green state has an excitation peak at 490 nm and an emission peak at 507 nm. The photo-activatable red state has an excitation peak at 553 nm and an emission peak at 573 nm. The number of activated fluorophores was controlled by varying intensity and illumination time of photoactivation. Activation intensities at 488 nanometer ranging from 10^{-6} to 10^{-3} W/cm^2 when applied for several milliseconds was sufficient to activate the accessible range of fluorophore densities for single-molecule microscopy from 0.01 to 1 molecules/μm^2.
We have created a 3T3 fibroblast cell line that expresses actin-Dendra2. Individual Dendra2 proteins and small clusters are observed as speckles by which location is analyzed to an accuracy of tens of nanometers at video-rate. Successively Fluorescence Speckle Microscopy (FSM) was employed to quantify the movement and (dis-)assembly dynamics of the actin cytoskeleton.

2412-Pos Board B398
Modeling Fission-Yeast Growth Partitioning and Oscillating Cortical Cdc42 Populations
Tyler Drake, Maitreyi Das, Peter Buchwald, Fulvia Verde, **Dimitrios Vavylonis**.
Polarity proteins mark two distinct, opposite regions of the fission-yeast cell membrane. These regions provide sites for growth, and maintaining them allows the cell to keep its elongated shape over generations. We present evidence that these regions compete for Cdc42, a growth precursor. A nonlinear-dynamics model divides the Cdc42 population into three subpopulations–one at each tip, one in the cytoplasm–and describes Cdc42 distributions by stable steady states of the dynamical system. This model describes this competition with differential equations that include autocatalytic accumulation and saturation of Cdc42 at the cell tips. According to this model, short cells must be monopolar and long cells must be bipolar, but cells of intermediate length could be either_-stable monopolar and bipolar steady states coexist for intermediate lengths. However these divisions depend on parameters, as illustrated by a phase diagram. In addition to its polarization, we found that Cdc42 oscillates between tips. We present a delayed-differential-equation model that adds delayed amplification of dissociation at the tips, and show that the complete model survives at least three experimental challenges. First, we decrease the amount of Cdc42 by removing its effector, Gef1; this leads to later bipolarity. Second, we probe the coexistence region with actin-depolymerizing drug Latrunculin A; this shows that longer monopolar cells can become bipolar following a disturbance. Third, we calculate lower cross-correlations between tips in longer mutant cells; this suggests a decrease in competition's importance as cells grow further. We demonstrate possible effects of noise on this model and speculate on the competitive advantage gained despite the cost of maintaining active oscillations.

2413-Pos Board B399
Quantitative Image Analysis and Modeling of Actin-Dependent Chromosome Transport in Starfish Oocytes
Nilah Monnier, Masashi Mori, Nathalie Daigle, Jan Ellenberg, Peter Lenart, **Mark Bathe**.
A dynamic filamentous actin (F-actin) meshwork in the nuclear region of starfish oocytes is essential for chromosome transport to the spindle pole during meiosis. To understand the molecular mechanism by which this meshwork transports chromosomes, we have developed computational image processing methods to analyze high spatiotemporal resolution timelapse movies of the dynamics of chromosomes, inert beads, and F-actin filaments in living cells during the congression process. A novel method for reliable 3D tracking of dynamic and amorphously-shaped chromosomes extracts sub-pixel resolution chromosome (and bead) trajectories with high temporal sampling, permitting analysis of their motion at a range of timescales. On long timescales chromosomes and beads exhibit directed motion with velocities that increase linearly with their starting distance from the spindle pole, consistent with a model of congression in which chromosomes are embedded in a homogeneously contracting meshwork of contractile units anchored at the spindle pole. We use Bayesian hypothesis testing to evaluate competing models of diffusive motion superimposed on advective transport and find that on short timescales chromosomes and beads exhibit confined/anomalous diffusion, suggesting that they are transported by passive diffusion inside confined spaces within the actively flowing actin meshwork. Finally, we use spatiotemporal image-correlation spectroscopy (STICS) to probe the spatiotemporal dynamics of the actin meshwork flow field surrounding the choromosomes, revealing changes in the actin meshwork velocity profile over time during the transport process. These quantitative analyses support a novel chromosome transport mechanism in which transport is accomplished by confinement of diffusing chromosomes within a dynamic actin meshwork that is organized into contractile units.